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See Note to File Attached string superseded by 1989 Fevice ACC#: 407571-01

DATA EVALUATION RECORD

1. CHEMICAL:

DEF

2.

TEST MATERIAL: Tribufos Technical (99% a.i.); Reference No. 85-R-26-39. Assigned Wildlife Int. Ltd. i.d. No. WIL-1161, upon receipt. Purity was reported as 98.7%.

STUDY TYPE: 3.

Avian Reproduction - Bobwhite Quail

Species Tested: Colinus virginianus

CITATION: Beavers, J. B., G. Marselas and M. J. Jaber. 1988. 4. DEF: A One-Generation Reproduction Study with the Bobwhite (Colinus virginianus). July 6. EPA Guideline No. 71-4. Project No. 149-127. Mobay Report No. 97437. 101 pp. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Mobay Corp., Stilwell, Kansas.

5. REVIEWED BY:

Jeffrey L. Lincer, Ph.D.,

Eco-Analysts, Inc.

Sarasota, FLorida

Signature:

Date: 10/10/88

6. APPROVED BY:

> James R. Newman, Ph.D., Proj. Mgr., KBN Engineering and Applied Sciences, Inc.

Signature: Joseph R. Human Date: 10/27/88

Henry T. Craven

Chief EEB/HED

**USEPA** 

Signature:

Date:

7. CONCLUSIONS The study is scientifically sound and, basically, meets the guidelines. Dietary concentrations of DEF at 148 ppm did not result in treatment related mortality, overt signs of toxicity or effects upon reproductive performance among adult bobwhite during the 20 week exposure period. At 148 and 262 ppm, there was a (nonsignificant) reduction in the numbers of eggs laid, and there was a statistically significant (P<.05) decrease in



egg shell thickness. At the 392 ppm concentration, there may have been one treatment related mortality. There were pronounced effects upon reproduction parameters and body weight gain, by 14-day survivors, at that concentration. The no-observed-effect concentration for bobwhite in this study was 148 ppm.

- 8. RECOMMENDATIONS: Applicant should respond to points raised in sections 14A and 14C.
- 9. BACKGROUND: N/A
- 10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: N/A
- 11. MATERIALS AND METHODS (PROTOCOLS):
  - A. Test Animals: Pen-reared bobwhite that were apparently healthy and phenotypically indistinguishable from wild birds, were purchased from Sand Prairie Quail Farm, Maquoketa, Iowa. All birds were from the same hatch and were 20 weeks of age at test initiation (first day of exposure to test diet). The birds were approaching their first breeding season and had not been used in previous testing. At test initiation, all birds were examined for physical injuries and general health. Birds that did not appear healthy were discarded. Sex of the birds was determined by a visual examination of the feather coat.

Adult birds were identified by individual leg bands. All eggs laid during the study were marked with a permanent ink marking pen for identification. Hatchlings were identified by leg bands so that the chicks could be traced to their parental pen of origin.

### B. Dosage and Design:

Study Phases The primary phases of the study and their approximate durations were:

- Acclimation 4 weeks.
- 2. Pre-photostimulation 8 weeks.
- Pre-egg laying (with photostimulation) 4 weeks.
- 4. Egg laying 9 weeks.
- Post-adult sacrifice (final incubation, hatching, and 14-day offspring rearing period) - 6 weeks.

#### Treatment Groups

Treatment levels were based upon known toxicity data and consultation with the clients. One hundred and twenty-eight (128) bobwhite (64 cocks and 64 hens) were randomly distributed into the below four groups.

| DEF           | (ppm)                 |         |       |         |
|---------------|-----------------------|---------|-------|---------|
| Nominal       | Mean Measured         | Number  | Birds | Per Pen |
| Concentration | Concentration         | Of Pens | Cocks | Hens    |
|               |                       |         |       |         |
| 1 - Controls  | ND                    | 16      | 1     | 1       |
| 2 - 150       | 148 + 11.0            | 16      | 1     | 1       |
| 3 - 280       | 262 + 25.5            | 16      | 1     | 1       |
| 4 - 410       | 392 <del>+</del> 23.3 | 16      | 1     | 1       |

### Procedure

Each group contained sixteen pairs of birds (one male and one female per pen). Each of three groups were fed diets containing nominal concentrations of either 150, 280 or 410 parts per million (ppm) of DEF, respectively or mean measured concentrations of 0, 148, 262 or 392 ppm. The fourth group was fed control diet containing an amount of the solvent (acetone) and carrier (corn oil) equivalent to the amount in the treated diets. Each of the four groups of adult birds was fed the appropriate diet from the initiation of the test until the terminal sacrifice.

The test birds were acclimated to the facilities for 4 weeks prior to initiation of the test. During acclimation and upon initiation of the study, the birds were maintained under a photoperiod of eight hours of light per day. During Week 8 the photoperiod was increased to seventeen hours of light per day to induce egg laying. The photoperiod was maintained at 17 hours of light per day until adult sacrifice. The first eggs were set for incubation during Week 13.

All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and observations. All birds that died during the study were necropsied. In addition, at the conclusion of the adult exposure period all birds were sacrificed by cervical dislocation, necropsied and disposed of by incineration.

Adult body weights were measured at study initiation, on Weeks 2, 4, 6, 8 and at terminal sacrifice. Body weights were not measured during egg laying because of the possible adverse effects handling may have on egg production. Feed consumption was measured for each pen for a seven day period every week throughout the study.

Eggs were collected daily from all pens, marked according to the pen of origin and stored in a cold At weekly intervals all eggs room until incubated. were removed from the cold room, counted and taken for egg shell thickness measurement. The remaining eggs were candled with a Speed King egg candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were discarded. All eggs that were not cracked or used for egg shell thickness measurements were placed in a Petersime Incubator. Eggs were candled again on Day 11 of incubation to determine embryo viability; and on Day 21 to determine embryo On Day 21 of incubation, the eggs were survival. placed in a Petersime Hatcher and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by pen.

All hatchlings, unhatched eggs and egg shells were removed from the hatcher on Day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded for identification by pen of origin and then housed according to the appropriate parental concentration grouping in brooding pens until 14 days of age. At 14 days of age the average body weight by parental pen of all surviving chicks was determined. The chicks were sacrificed with chloroform or carbon dioxide and disposed of by incineration.

weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during odd numbered weeks (1,3,5, etc.) and from each of the even numbered pens during the even numbered weeks (2,4,6, etc.). The eggs were opened, the contents removed, and the shell thoroughly washed. The shells were then allowed to air dry for at least one week at room temperature. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.005 mm.

Records were maintained by pen for each of the following reproductive parameters:

- Eggs Laid

- Eggs Cracked

- Eggs Set

- Viable Embryos

- Live 3-week Embryos

- Hatchlings

- Body Weight of Hatchlings

- 14-Day Old Survivors - Eggshell Thickness

- Body Weight of 14-Day Old Survivors

### Animal Diet

Basal diet for the adult birds and their offspring was formulated to Wildlife International Ltd. specifications by Agway, Inc. The diet contained 28% protein minimum, 2.5% fat minimum and 5% fiber maximum. Water was supplied by the town of Easton. Feed and water are analyzed periodically as per Wildlife International Ltd. SOP No. 4.7. Neither the adults nor offspring received any form of medication during the study.

The adults were fed a game bird ration formulated for breeding birds. During the study the birds received the appropriate test or control diet from study initiation to terminal sacrifice. Water and feed were provided ad libitum during acclimation and during the test.

All offspring received a game bird ration formulated for young growing birds (identical to adult diet, but without the addition of limestone). The test substance was not mixed into the diet of the offspring. All offspring received a water soluble vitamin and electrolyte mix in their water from the day of hatch until the birds were 14 days of age. Feed and water were provided to the offspring ad libitum.

#### Diet Preparation

Test diets were prepared by mixing DEF into a premix which was used for weekly preparation of the final diet. Control diet and three test concentrations (150, 280 and 410 ppm nominal) were prepared weekly beginning on August 6, 1987 and presented to the birds on Thursday of each week. When necessary during the study, additional feed was prepared. Dietary concentrations were adjusted for purity of the test substance and are presented as ppm of active ingredient.

## Diet Analysis

Samples of the control diet and each of the test diets were taken weekly after mixing and frozen immediately after collection. Stability and homogeneity samples also were collected. Samples were shipping to Mobay Corporation for analysis of the active ingredient.

## Housing and Environmental Conditions

The adult birds were housed indoors in batteries of pens manufactured by Georgia Quail Farm Manufacturing measuring approximately 30 x 51 cm. The pens had sloping floors which resulted in ceiling height ranging from 21 to 26 cm. The pens were constructed of galvanized wire grid and galvanized sheeting.

Each pen was equipped with a feeder. Each week, sufficient feed for seven days was placed in feeders for each pen and presented to the birds. During the week, additional feed was added to the feeders where excessive wastage by the birds made it necessary. Waterers were changed and water added as necessary to provide potable water (generally every one or two days).

The birds were maintained in a separate study room which helped avoid excessive disturbances. The average temperature in the adult bobwhite study room during the course of the study was  $22.3^{\circ}\text{C} + 2.4^{\circ}\text{C}$  (SD) with an average relative humidity of  $5\overline{3}\%$ . The air handling system in the study room was designed to vent up to fifteen room air volumes every hour and replace it with fresh air.

The photoperiod in the adult bobwhite study room was maintained by a time clock. The photoperiod for the first 7 weeks of the study was eight hours of light per day. The photoperiod was then increased during Week 8 to seventeen hours of light per day and was maintained at that length until sacrifice of adult birds. The birds received approximate 129 lux (12 footcandles) of illumination throughout the study, provided by Chroma 50 fluorescent lights which closely approximate noon-day sunlight.

Hatchlings were placed in batteries of brooding pens manufactured by Beacon Manufacturing. Each pen measured approximately 72 x 90 x 23 cm high. The external walls and ceilings of each pen were constructed of galvanized wire mesh and galvanized sheeting. Floors were of galvanized wire mesh. Thermostats in the brooding compartment of each pen were set to maintain a temperature of approximately 38°C from the time of hatching until the birds were 14 days of age. The photoperiod for the hatchlings was maintained by a time clock at 16 hours of light per day.

Housing and husbandry practices were conducted so as to adhere to the "Guide and Care and Use of Laboratory Animals," NIH Publication No. 85-23, 1985.

# Incubation and Hatching

Eggs were collected daily and stored in a cold room at 11.2°C+1.2°C (SD) and approximately 75% mean relative humidity. All eggs to be incubated were fumigated with formaldehyde gas to reduce the possibility of pathogen contamination prior to incubation.

Eggs were set for incubation on a weekly basis. The eggs were placed in the incubator where the temperature was maintained at  $37.4^{\circ}\text{C} + 0.1^{\circ}\text{C}$  (SD) with a wet bulb temperature of  $29.3^{\circ}\text{C} + \overline{0.9^{\circ}\text{C}}$  (SD) (mean relative humidity of approximate  $\overline{56}\%$ ). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotating device, designed to rotate the eggs from 50° off of vertical in one direction to 50° off of vertical in the opposite direction (total arc of rotation is 100°) each hour through Day 21 of incubation. The eggs were transferred to the hatcher on Day 21. Eggs were not rotated in the hatcher. The temperature in the hatcher was  $37.1^{\circ}C + 0.2^{\circ}C$  (SD) and the wet bulb temperature was raised to 32.5°C + 1.2°C (SD) (mean relative humidity of 70%).

C. Statistics: Upon completion of the study, Dunnett's method (1,2) was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group.

Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data. Each of the following parameters was analyzed statistically:

Adult Feed Consumption
Adult Body Weight
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-week Embryos of
Viable Embryos
Hatchlings of 3-week
Embryos 12.

Offspring's Body Weight
Hatchlings of Eggs Set
Hatchlings of Maximum Set
14-Day Old Survivors of
Maximum Set
14-Day Old Survivors of
Hatchlings
Eggshell Thickness

#### REPORTED RESULTS:

# "Results of Diet Analysis

Samples of test diet fed to bobwhite were analyzed for DEF. The results showed values that ranged from 83% to 105% of nominal with an average of 96%. Nominal and mean measured concentrations were as follows:

| DEF           | (ppm)          |
|---------------|----------------|
| Nominal       | Mean Measured  |
| Concentration | Concentration  |
| 0             | ND*            |
| 150           | 148 + 11.0     |
| 280           | 262 + 25.5     |
| 410           | $392 \mp 23.3$ |

<sup>\*</sup> Not Detectable

## "Mortalities

No mortalities occurred during the course of the study in the control group or the [measured] 262 ppm treatment group. Three incidental mortalities occurred in the 148 ppm treatment group, and three mortalities occurred in the 392 ppm treatment group. Two of the mortalities at 392 ppm were incidental to treatment and one mortality may have been treatment related.

The first mortality from the 148 ppm treatment group was a hen...found dead during Week 7. She had been observed the day prior to death with her toe caught in the side wall of the pen. Necropsy revealed a bird in good flesh and

feather coat, with a cut on the third toe of the right foot. Small areas of bruising were noted on the midline of the cranium and the dorsal surface of the neck. lesions were noted. An additional hen...was first observed at the beginning of Week 12 with head lesions, which became more pronounced and were accompanied by lethargy prior to death. Necropsy revealed a bird light in body weight (166g) No internal lesions were with extensive head lesions. observed. The third mortality in the 148 ppm treatment group was a hen found dead at the beginning of Week 16. hen had not exhibited prior clinical signs, was moderate in body weight and showed evidence of feather loss on the back. Internally, extensive egg yolk peritonitis was observed. Necropsy of the pen mates of all three birds found dead at the 148 ppm treatment group were unremarkable.

The first mortality at 392 ppm was a hen...that severe broken leg during body weight sustained a measurements at the end of Week 2 and was euthanized. second mortality was a hen...that was first observed at the beginning of Week 10 exhibiting a ruffled appearance and wing droop. This bird also displayed depression and reduced reaction to external stimuli (sound and movement) prior to death during Week 10. Necropsy revealed an extremely emaciated bird (body weight - 110 g) with feather loss on the back of the head. Internally, the ceca were distended with fluid and gas. No other gross lesions were observed. The test substance appeared to have been at least contributory to that mortality (see Gross Necropsy section). The final mortality in the 392 ppm treatment group was a cock...found dead during Week 11. He had been noted earlier in the week with head lesions and displaying lethargy and a ruffled appearance. Gross necropsy revealed extensive head lesions with bone exposed on the cranium. No other lesions were noted. The necropsy of the pen mates of these birds was unremarkable.

#### "Clinical Observations

No overt signs of toxicity were observed at any concentration tested. Respiratory signs such as coughing were observed in one cock and three hens from the 0 ppm group, two cocks and one hen from the 148 ppm group, two cocks and two hens from the 262 ppm group, and two cocks and six hens from the 392 ppm group. Those signs, which were first observed during Week 4, were apparent through Week 7 and had abated by Week 8.

Initial examination of birds from this lot by the Health Laboratory, Maryland Department Agriculture, Salisbury MD...was negative for pathogens. Examination of extra birds from this same lot submitted on 187 August 17, showed that two of six birds were seropositive for adenovirus. Ultimate isolation of an adenovirus from tissue culture of the birds was completed on September 3, 1987. A diagnosis of quail bronchitis was Since this disease is generally self-limiting in adult birds, no attempts were made to treat the affected birds, all of which recovered uneventfully.

Other clinical signs not attributed to treatment, such as intermittent lethargy or depression, reduced reaction to external stimuli (sound and movement), ruffled appearance, wing droop or head curl, were observed in a few birds in all treatment groups during the course of the study. Except for the mortalities and clinical signs previously noted, and aside from lesions or observations normally associated with pen wear and/or interaction among pen mates, all other birds at all concentrations appeared normal throughout the study.

# "Gross Necropsy

All surviving adults were necropsied at adult terminal sacrifice. All lesions observed in the 148 ppm and 262 ppm treatment groups were considered to be incidental and not related to treatment. All birds necropsied from the 392 ppm treatment groups were noted to have a slight to moderate distention of the gastrointestinal tract. There also appeared to be an increase in the number of hens with a regressing or regressed ovary at the 392 ppm test concentration....

# "Adult Body Weight and Feed Consumption

When compared to the control group, there were no apparent treatment related effects upon body weight among adult birds at any concentration tested. There were no statistically significant differences between the control group and the 148, 262 or 392 ppm groups at any body weight interval....

Due to excessive wastage by some birds, feed consumption was variable between pens. There were no apparent treatment related effects upon feed consumption among birds at the 148 and 262 ppm concentrations. When compared to the control group, there were no statistically significant differences between the control group and the 262 ppm groups during the study. At 148 ppm during Weeks 16, 17, 18, 19 and 20, there was a slight, but statistically

significant (P<.01), increase in feed consumption when compared to the control group that was considered incidental to treatment. At 392 ppm, there was a slight but statistically significant increase in feed consumption during Weeks 5, 6, 14 and 18 at P<.05 and during Weeks 7, 11, 13, 15, 16, 17, 19 and 20 at P<.01. These increases, particularly those that occurred during the early portion of the study, may have been treatment related....

# "Reproductive Results

There were not apparent treatment related effects upon reproductive parameters at 148 ppm. When compared to the control group, at 148 ppm there was a...statistically significant decrease in the number of cracked eggs as a percentage of eggs laid. At the 262 ppm concentration, there may have been a slight reduction in the number of eggs laid, although the difference was not statistically significant. There was no apparent effect upon any other reproductive parameter at the 262 ppm concentration.

At 362 ppm, there was a marked effect upon reproductive performance with statistically significant (P<.01) effects upon the numbers of eggs laid, the number of 14-day old survivors as a percentage of the number of hatchlings, the number of hatchlings as a percentage of the number of eggs set; and the number of 14-day old survivors as a percentage of the number of eggs set. While not statistically significant, there was also a reduction in the number of viable embryos as a percentage of eggs set.

Due to the inadvertent failure of one brooder on January 26, 1988, incidental mortalities of offspring occurred. Fifteen offspring from the 148 ppm group, 18 offspring from the 262 ppm group, and two offspring from the 392 ppm group were found dead. These mortalities were not considered when computing the percentage of 14-day old survivors....

## REPRODUCTIVE DATA

|                          | 0   | GROUPS<br>150 | (PPM)<br>280 | 410 |
|--------------------------|-----|---------------|--------------|-----|
| There Tail 3             |     |               |              |     |
| Eggs Laid                | 633 | 518           | 518          | 181 |
| Eggs Cracked             | 6   | 0             | 1            | 4   |
| Eggs Set                 | 563 | 466           | 457          | 137 |
| Viable Embryos           | 480 | 381           | 372          | 101 |
| Live 3-week Embryos      | 476 | 378           | 370          | 99  |
| Hatchlings               | 427 | 335           | 335          | 84  |
| 14-Day Old Survivors     | 377 | 289           | 277          | 51  |
| Eggs Laid/Hen            | 40  | 40            | 32           | 14  |
| Eggs Laid/Hen/Day        | .61 | .61           | .50          | .21 |
| 14-Day Old Survivors/Hen | 24  | 22            | 17           | 4   |

@ - Based on 65 days.

# REPRODUCTIVE DATA - PERCENT

|                               |     | GROUPS | (PPM) |      |
|-------------------------------|-----|--------|-------|------|
|                               | 0   | 150    | 280   | 410  |
| Eggs Laid                     | 633 | 518    | 518   | 181  |
| Eggs Laid/Max. Laid (%)       | 63  | 63     | 51    | 22** |
| Eggs Cracked/Eggs Laid (%)    | 1   | 0*     | 0     | 2    |
| Viable Embryos/Set (%)        | 84  | 83     | 80    | 69   |
| Live 3-Week Embryos/Viable    | 99  | 99     | 100   | 99   |
| Hatchlings/3-Week (%)         | 90  | 88     | 88    | 85   |
| 14-Day Old Survivors/Hatch    | 88  | 91     | 86    | 56** |
| Hatchlings/Set (%)            | 75  | 73     | 72    | 59   |
| 14-Day Old Survivors/Set      | 66  | 66     | 60    | 29** |
| Hatchlings/Max. Set (%)       | 47  | 45     | 37    | 11** |
| 14-Day Old Surv./Max. Set (%) | 41  | 39     | 30    | 7**  |

<sup>\*</sup> Difference from the control statistically significant at P<.05.

<sup>\*\*</sup> Difference from the control statistically significant at P<.01.

# "Egg Shell Thickness

There were no apparent treatment related effects upon egg shell thickness at 148 ppm. When compared to the control group, there were no statistically significant differences in egg shell thickness at 148 or 392 ppm. At 262 ppm, when compared to the control group, there was a slight but statistically significant (P<.05) decrease in mean egg shell thickness...

| EGG SHELL THIC                  | KNESS (1       | mm )           |                | ······································ |
|---------------------------------|----------------|----------------|----------------|--|
|                                 |                | DEF            | (PPM)          |  |
|                                 | 0              | 150            | 280            | 410                                    |
| No. of Eggs Measured            | 62             | 50             | 59             | 31                                     |
| Mean Egg Shell Thickness (mm)   | 0.227          | 0.219          | 0.212*         | 0.220                                  |
| <pre>+ standard deviation</pre> | <u>+</u> 0.015 | <u>+</u> 0.015 | <u>+</u> 0.015 | <u>+</u> 0.025                         |

<sup>\*</sup> Difference from the control statistically significant at P<.05.</p>

# "Offspring Body Weights

There were no apparent treatment related effects upon the body weights of hatchlings or 14 day old survivors at the 148 ppm or 262 ppm concentrations. While there were no statistically significant differences in the body weights of hatchlings at 393 ppm, there was a slight but statistically significant (P<.01) decrease in the body weights of 14-day old survivors at 392 ppm..."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Dietary concentrations of DEF at 148 ppm did not result in treatment related mortality, overt signs of toxicity or effects upon reproductive performance among adult bobwhite during the 20 week exposure period. At 262 ppm, there may have been a slight reduction in the numbers of eggs laid, and there was a slight but statistically significant (P<.05) decrease in egg shell thickness. At the concentration, there may have been one treatment related effects pronounced and there were reproductive parameters at that concentration. The noobserved-side-effect concentration for bobwhite in this study was 148 ppm."

"This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160. The final report was determined to be an accurate reflection of the results obtained. The dates of all audits and [the]...results of those audits were reported to the Study Director/Laboratory Management...."

A total of sixteen (16) audits were carried out during the experimental and report writing phases of this work.

# 14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

# A. Test Procedure(s):

(1) Raw data for mortality was not provided so it could not be used to confirm text.

Raw data for weight changes showed a consistent trend for females in higher dosage groups to gain less weight but this was not discussed in text.

Raw data for feed consumption, gross necropsy, eggshell thickness, and offspring body weights supported text.

Raw data for reproductive results supported text with the exception of number of eggs laid. Text indicated that, "There were no...treatment related effects upon reproductive parameters at 148 ppm...[but] at the 262 ppm concentration, there may have been a slight reduction in the number of eggs laid. Raw data revealed the same number (518) of eggs laid for both groups. In addition, text indicated that there was a statistically significant effect upon numbers of eggs laid in the 392 ppm group, but the relevant table (3) didn't provide any indication (i.e. a footnote) to this effect.

- (2) Study procedures were consistent with guidelines, with the following exceptions:
  - (a) Number of embryos that pipped shell (vs. embryos that liberated themselves) was not reported, as required by subdivision E.
  - (b) Basis for selection of dosages, especially as they might relate to expected environmental levels, was not clear.

- (c) Birds were maintained on a photoperiod of 8 hours of light per day (vs. 7 hours recommended by Subdivision E and the SEP) for the first eight weeks of study.
- (d) Birds received approximately 12 footcandles (vs. the 6 which is indicated as adequate by Subdivision E).
- (e) According to the authors, eggs were candled on Day 21 to determine embryo survival (vs. Day 18 recommended by the SEP).
- (f) Gross necropsy results didn't include observations on liver, kidney, heart or spleen, as suggested by the SEP.
- B. Statistical Analysis: See attached printouts.
- Discussion/Results: Dietary concentrations of DEF at C. 148 ppm did not result in treatment related mortality, overt signs of toxicity or statistically significant effects upon reproductive performance among adult bobwhite during the 20 weeks exposure period. The 148 ppm diet, however, and the 262 ppm diet resulted in an 18% reduction in eggs laid. At 262 ppm (but not at 392 ppm) there was a statistically significant (P<.05) decrease in egg shell thickness. At the 392 ppm concentration, there may have been one treatment related mortality and there were pronounced effects upon reproductive parameters at that concentration. At this (i.e. 392 ppm) concentration, statistically significant (P<.01) effects were observed for: number of eggs laid; number of 14-day old survivors as percentage of number of hatchlings; the number of hatchlings as percentage of maximum number of eggs set; and the number of 14-day old survivors as percentage of number of eggs set and maximum number of eggs set. There was also a (non-statistically significant) reduction in number of viable embryos as a percent of eggs set. The no-observed-effect concentration for bobwhite in this study was 148 ppm, the lowest concentration tested.

The study is valuable but the following detract from that value:

(a) No clear basis for the chosen dosages, especially as they might relate to expected environmental concentrations, was provided.

- (b) Raw mortality data were not provided. Therefore, that information could not be used to confirm text assertions.
- (c) Although raw weight gain data revealed increased dosage, this was not discussed in the text.
- D. Adequacy of the Study:
  - (1) Classification: Supplemental
  - (2) Rationale: This study appears to be scientifically sound but apparent deviations from meeting guideline requirements detract from the study.
  - (3) Reparability: Should be reconsidered for elevation to Core status after applicant responds to points raised in sections 14A and 14C.
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on 10/10/88
- 16. CBI APPENDIX: N/A

### REFERENCES

- Dunnett, C. W., "A Multiple Comparison Procedure for Comparing Several Treatments with a Control", <u>Jour. Amer.</u> Statis. Assoc. 50:1096-1121, 1955.
- Dunnett, C. W., "New Tables for Multiple Comparisons with a Control", <u>Biometrics</u> 20:482-491, 1964.

## ONE LINER SHEET

| Shaughnessey No                  |                    | Chemical Name | <u>DEF</u> | Chemical | Class | <br>Page | of                |                      |
|----------------------------------|--------------------|---------------|------------|----------|-------|----------|-------------------|----------------------|
| Study/Species/Lab<br>Accession # | Chemical<br>% a.i. |               | Resu       | lts      |       | -        | Reviewer/<br>Date | Validation<br>Status |

| Anian Dannadustian   |     | Group         | Dose (ppm) | Affected/Parameters | Mort.(%) | % CHe Inh. |          |              |
|--|-----|---------------|------------|---------------------|----------|------------|----------|--------------|
| Avian Reproduction<br>Species: Bobwhite Quail                    |     | Control       | 0          | 0/ all              |          | N/A        |          |              |
|  | 99. | Treatment I   | 150        | *                   | 9        | N/A        | Lincer/  | Cumalamental |
| Lab: Wildlife Int. Ltd.<br>Project #: 149-127<br>AC #: 407571-01 |     | Treatment II  | 280        | *                   | 0        | N/A        | 10-10-88 | Supplemental |
|  |     | Treatment III | 410        | t                   | 9        | N/A        |          |              |

Study Duration: 20 week exposure

Comments: \* 18% reduction in eggs laid in both Treatments I and II.

t Clear impacts on reproductive parameters (i.e. eggs laid, 14-day survivors/max. set hatchlings and egg set, hatchlings/max. set), viable embryos, live 3-week embryos, and weight gain of 14-day survivors.

Note to File:

The 5-89 DER takes precedent over the 88 review.

NOTE: KBN reviewed the same study twice & found different results: 10.27.88 Review - supplemental with possible upgrade to core

5.06 89 Review - CORE

The reviewer determined we would

90 with the 2nd, or 5.26.89

review by LBN.

numa Cok